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(71) Applicant: PROCEPT, INC. [US/US]; 840 Memorial Drive, Cambridge, MA 02139 (US).

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(54) Title: METAL-CONTAINING COMPOUNDS AND THEIR USE FOR INHIBITING THE IMMUNE RESPONSE

(57) Abstract

Use of compounds as immunosuppressive agents to prevent or significantly reduce graft rejection in organ and bone marrow transplantation is described. The compounds described herein can also be used as immunosuppressant drugs for T-lymphocyte mediated autoimmune diseases, such as diabetes, and may be useful in alleviating psoriasis and contact dermatitis. The compounds can also be used therapeutically in the treatment of hyperproliferative vascular disease and to reduce/suppress the immune response in a mammal undergoing gene therapy.

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-438756	31-07-91	DE-A-	4000154	11-07-91
		CA-A-	2033249	05-07-91
		DE-D-	59005456	26-05-94
		JP-A-	4305535	28-10-92
		JP-B-	8013756	14-02-96
		US-A-	5578571	26-11-96
US-A-5512687	30-04-96	AU-A-	4017695	23-05-96
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K33/24 A61K33/32 A61K33/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 438 756 (SYMBIOTEC) 31 July 1991 see page 2 ---	1,2,11, 12
E	US,A,5 512 687 (BASTOS) 30 April 1996 see the whole document -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Klaver, T

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(54) Title: METAL-CONTAINING COMPOUNDS AND THEIR USE FOR INHIBITING THE IMMUNE RESPONSE

(57) Abstract

Use of compounds as immunosuppressive agents to prevent or significantly reduce graft rejection in organ and bone marrow transplantation is described. The compounds described herein can also be used as immunosuppressant drugs for T-lymphocyte mediated autoimmune diseases, such as diabetes, and may be useful in alleviating psoriasis and contact dermatitis. The compounds can also be used therapeutically in the treatment of hyperproliferative vascular disease and to reduce/suppress the immune response in a mammal undergoing gene therapy.

wherein Bipy is 2,2'-bipyridine, MeIm is methylimidazole, en is ethylenediamine, py is pyridine and OTf is triflate.

12. The compound, composition or use according to any one of Claims 1 to 11 wherein the autoimmune disease is selected from the group consisting of graft rejection, insulin dependent diabetes mellitus, rheumatoid arthritis, psoriasis, hyperplasia of the epidermis, contact dermatitis and symptoms associated therewith, steroid resistant asthma, multiple sclerosis and lupus erythematosus.

13. A method for (i) preventing or substantially reducing a T-lymphocyte mediated immune response of a mammal (e.g., an autoimmune disease), (ii) treating hyperproliferative vascular disorers or (iii) reducing or suppressing an immune response of a mammal undergoing gene therapy comprising administering to a mammal a therapeutic amount of the compound or composition according to any one of Claims 1, 2, 4 to 12.

imidazole; and wherein R is a linear or branched alkyl of 1 to 8 carbon atoms or aryl.

10. The composition or use of the composition according to any one of Claims 2 to 9 further comprising a steroid and/or an immunosuppressant selected from the group consisting of cyclosporin, rapamycin, FK-506, azathioprine, mizoribine, mycophenolate mofetil, brequinar sodium, leflunomide and 15-deoxyspergualin.

11. The compound, composition or use according to any one of Claims 1 to 3 wherein the complex is selected from the group consisting of:

$[Co(NH_3)_6]Cl_3$;
$[Co(NH_3)_5Cl]Cl_2$;
$[Os(NH_3)_5(H_2O)]Cl_3$;
$[Os(NH_3)_5(N_2)]Cl_2$;
$(NH_4)_2OsCl_6$;
$[Co(NH_3)_5(py)]Cl_3$;
$(NH_4)_2PtCl_6$;
cis-$Pt(NH_3)_2Cl_2$;
$[Pt(NH_3)_4]Cl_2$;
$[Os(Bipy)_3]Cl_3$;
$[Co(1\text{-}MeIm)_6](OTf)_2$;
$[Co(en)_3]Cl_3$;
$[Pd(NH_3)_4]Cl_2$;
$[Rh(NH_3)_5Cl]Cl_2$;
$[Ir(NH_3)_5Cl]Cl_2$;
$(NH_4)_3RhCl_6$;
$(NH_4)_3IrCl_6$;
$[Rh(NH_3)_6](OTf)_3$;
$[Rh(NH_3)_5(py)](OTf)_3$; and

1,2-bis(dimethylphosphino)methane; carboxylates; and bis-phosphines.

6. The compound, composition or use according to Claim 5 wherein T is an aliphatic amine or aromatic amine.

7. The compound, composition or use according to Claim 6 wherein T is selected from the group consisting of diethylenetriamine, dipropylenetriamine, 1,4,7-triazacyclononane, 1,4,7-triazacyclodecane, 2,2',6",2"-terpyridine, bis-(2-pyridylmethyl) amine, bis-(2-imidazolylmethyl)amine, potassium tris-pyrazolyl borate, tris-pyrazolyl methane, iminodiacetic acid, nitrilotriacetic acid, triethanol amine, bis-(3-methylphenoxy)amine, 1,4,7-trithiacyclononane, 1,4,7-trithiacyclodecane and 2-(arylazophenyl)thioether.

8. The compound, composition or use according to Claim 7 wherein P is selected from the group consisting of 1,4,7,10-tetrazacyclododecane,1,4,8,11-tetraazacyclotetradecane, tris-(2-pyridylmethyl)amine, tris-(2-imidazolylmethyl)amine, 1,4,8,11-tetrathiacyclotetradecane, 1,4,7,10-tetrathiacyclotridecane, α,α'-bis-(bis-(2-diphenylphosphinoethyl)amino)ethane and α,α'-bis-(bis-(2-diphenylphosphino)-m-xylene.

9. The compound, composition or use according to Claim 8 wherein Z is a counterion selected from the group consisting of F^-, Cl^-, Br^-, I^-, NO_3^-, NH_4^+, NR_4^+, PF_6^-, BPh_4^-, SO_4^{-2}, S_8^{-2}, $S_2O_7^{-2}$, $CF_3SO_3^-$, BF_4^-, H^+, Na^+, K^+, ClO_4^-, acetate, lactate, citrate, tartarate, succinate, maleate, malonate, gluconate, hydrochloride, hydrobromide, phosphorate, nitrate, sulfate, trifluoromethansulfonate, methanesulfonate, and $RImH^+$, where Im is

containing ligands, sulfur containing ligands and phosphorus containing ligands; and

wherein when the complex is charged, then Z is at least one counterion of appropriate charge to render the overall charge of the complex neutral.

3. Use of a compound or composition according to Claims 1 or 2 for the manufacture of a medicament for (i) treating or substantially reducing a T-lymphocyte mediated immune response of a mammal (e.g., an autoimmune response), (ii) treating hyperproliferative vascular disorders or (iii) reducing or suppressing an immune response of a mammal undergoing gene therapy.

4. The compound, composition or use according to Claim to any one of Claims 1 to 3 wherein L is selected from the group consisting of imidazole, pyridine, ammonia, triazole, pyrazole, quinoline, pyrazine, pyridazine, pyrimidine, quinoxaline, quinazoline, piperidine and their derivatives obtained by substituting for one or more hydrogen atoms with one or more of the following moieties C1-C8 alkyl, C2-C6 alkenyl, hydroxyl, nitro, amino, carboxyl, ester, aliphatic and aromatic amines, phosphine, phosphite, thiolate, sulfoxide, alkoxide, phenolate, carboxylic acids, C3-C8 cyclic alkyl, aryl or substituted aryl, carboxylate, C1-C8 linear, branched or cyclic amine, carbonyl, thio, phenyl, benzyl, imidazole and combinations thereof.

5. The compound, composition or use according to Claim 4 wherein B is selected from the group consisting of ethylenediamine, propylenediamine, 1,2-cyclohexanediamine and the corresponding alkylated amines thereof; 2,2'-bipyridine, 1,10-phenanthroline; 2-aminopicoline; potassium-bis-pyrazolyl borate, bis-pyrazolyl methane;

platinum, rhenium, cobalt, osmium, manganese, copper, nickel and rhodium, having an oxidation state of 2, 3 or 4 depending on the nature of M;

wherein the number of coordinating atoms is 4 or 6 depending on the oxidation state and the nature of M;

wherein when the number of coordinating atoms is 4 then

l is 0, 1, 2, 3 or 4;
b is 0, 1 or 2;
t is 0 or 1;
p is 0 or 1;

wherein when the number of coordinating atoms is 6 then

l is 0, 1, 2, 3, 4, 5 or 6;
b is 0, 1, 2 or 3;
t is 0, 1 or 2;
p is 0 or 1;

wherein each L is independently a monodentate ligand selected from the group consisting of nitrogen containing ligands, phosphorus containing ligands, sulfur containing ligands, carbon containing ligands, oxygen containing ligands and halide;

wherein each B is independently a bidentate ligand selected from the group consisting of aliphatic amines, heterocyclic aromatic amines, sulfur containing ligands, carbon containing ligands, oxygen containing ligands and phosphorus containing ligands;

wherein each T is independently a tridentate ligand selected from the group consisting of nitrogen containing ligands, sulfur containing ligands, carbon containing ligands, oxygen containing ligands and phosphorus containing ligands;

wherein each P is independently a polydentate ligand selected from the group consisting of nitrogen containing ligands, oxygen containing ligands, carbon

wherein each L is independently a monodentate ligand selected from the group consisting of nitrogen containing ligands, phosphorus containing ligands, sulfur containing ligands, carbon containing ligands, oxygen containing ligands and halide;

wherein each B is independently a bidentate ligand selected from the group consisting of aliphatic amines, heterocyclic aromatic amines, carbon containing ligands, sulfur containing ligands, oxygen containing ligands and phosphorus containing ligands;

wherein each T is independently a tridentate ligand selected from the group consisting of nitrogen containing ligands, sulfur containing ligands, carbon containing ligands, oxygen containing ligands and phosphorus containing ligands;

wherein each P is independently a polydentate ligand selected from the group consisting of nitrogen containing ligands, oxygen containing ligands, carbon containing ligands, sulfur containing ligands and phosphorus containing ligands; and

wherein when the complex is charged, then Z is at least one counterion of appropriate charge to render the overall charge of the complex neutral.

2. A composition comprising a physiologically acceptable vehicle and an immunosuppressive amount or an antiproliferative amount of a compound having the general formula:

$$[ML_lB_bT_tP_p]Z$$

and physiologically acceptable salts thereof;

wherein M is selected from the group consisting of iridium, palladium, chromium, iron, technetium,

CLAIMS

We claim:

1. A compound for (i) preventing or substantially reducing a T-lymphocyte mediated immune response of a mammal (e.g., an autoimmune disease), (ii) treating hyperproliferative vascular disorers or (iii) reducing or suppressing an immune response of a mammal undergoing gene therapy, said compound having the general formula:

$$[ML_lB_bT_tP_p]Z$$

and physiologically acceptable salts thereof;

wherein M is selected from the group consisting of iridium, palladium, chromium, iron, technitium, rhenium, cobalt, osmium, manganese, copper, platinum, nickel and rhodium, having an oxidation state of 2, 3 or 4 depending on the nature of M;

wherein the number of coordinating atoms is 4 or 6 depending on the oxidation state and the nature of M;

wherein when the number of coordinating atoms is 4 then

l is 0, 1, 2, 3 or 4;
b is 0, 1 or 2;
t is 0 or 1;
p is 0 or 1;

wherein when the number of coordinating atoms is 6 then

l is 0, 1, 2, 3, 4, 5 or 6;
b is 0, 1, 2 or 3;
t is 0, 1 or 2;
p is 0 or 1;

Table (cont'd)

Examples	Formula	IC_{50} (μg/mL)
PIC 5751	$[Pd(NH_3)_4]Cl_2$	55
PIC 5752	$[Rh(NH_3)_5Cl]Cl_2$	
PIC 5753	$[Ir(NH_3)_5Cl]Cl_2$	
PIC 5754	$(NH_4)_3RhCl_6$	57
PIC 5755	$(NH_4)_3IrCl_6$	14
PRO 5767	$[Rh(NH_3)_6](OTf)_3$	20
PRO 5768	$[Rh(NH_3)_5(py)](OTf)_3$	>100

py = pyridine
OTf = triflate
MeIm = methylimidazole
en = ethylenediamine
Bipy = 2,2'-bipyridine

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims:

Tetanus toxoid (TT; Connaught Labs, Willow Dale, ON) was used as a stimulating antigen at a concentration of 5 LF/ml.

The test wells containing PBL's, were exposed to antigen, along with various dilutions of the solutions containing the compound, as shown in the Table.

Subsequently, TT antigen/compounds exposed PBL's were pulsed with 1 μCi/well of ^{3}H-thymidine on day 5 using a standard procedure known in the art. The cells were then harvested 16 hours later onto a glass fiber filter using a TOMTEC cell harvester. Thymidine incorporation was measured by liquid scintillation counting using a Beta plate counter (Pharmacia, Inc., Piscataway, N.J.).

The results of the assay are shown in the Table.

Table

Examples	Formula	IC_{50} (μg/mL)
PIC 2447	$[Co(NH_3)_6]Cl_3$	5
PRO 4319	$[Os(NH_3)_5(N_2)]Cl_2$	>1
PRO 4320	$[Co(NH_3)_5(py)]Cl_3$	11
PRO 4321	$[Co(NH_3)_5Cl]Cl_2$	>1
PRO 4757	$[Os(NH_3)_5(H_2O)]Cl_3$	>1
PIC 5025	$(NH_4)_2OsCl_6$	50
PIC 5027	$(NH_4)_2PtCl_6$	15
PIC 5028	cis-$Pt(NH_3)_2Cl_2$	1.3
PIC 5030	$[Pt(NH_3)_4]Cl_2$	>100
PRO 5286	$[Os(Bipy)_3]Cl_3$	35
PRO 5440	$[Co(1\text{-}MeIm)_6](OTf)_2$	6
PRO 5749	$[Co(en)_3]Cl_3$	>100

drug delivery to patients in both preclinical and clinical settings.

It is known that compounds having T lymphocyte immunosuppressive properties may also be useful in inhibiting the proliferation of cardiac smooth muscle cells. Based upon this, it is expected that the compounds can be used for the treatment of hyperproliferative vascular disorders, such as restenosis and atherosclerosis.

The invention also provides a method for coadministration of the compounds described herein with agents designed for gene therapy. It is believed that the use of the compounds of this invention may suppress the immune system and reduce/minimize an immune response against the gene delivery vehicle, so that therapeutic levels of transgene expression can be achieved in an animal or human. Any type of gene therapy delivery vehicle can be coadministered, such as those well known in the art. Concurrent adminstration of the gene therapy delivery vehicle and the compounds of this invention, either as a single unit dose or taken individually, is preferred.

The invention will be further illustrated by the following non-limiting Exemplification:

Exemplification

PBL Antigen Specific Proliferation Assay

The lymphocytes were prepared by first separating them from the blood samples of several donors by Ficoll gradient separation as described by standard procedure known in the art. The isolated lymphocytes were then grown in RPMI 1640 medium containing 5% human AB serum, glutamine (2mM), penicillin/streptomycin, 100 U/ml/100 μg/ml sodium pyruvate (1mM) and HEPES buffer (10mM).

For assay purposes, PBL's were incubated at a density of 10^5 per 200μl of medium per well of a 96-well plate.

The specific dosage level of active ingredient will depend upon a number of factors, including biological activity of the compound, age, body weight, sex, general health, severity of the particular disease to be treated and the degree of immune suppression desired, as well as appropriate pharmacokinetic properties. It should be understood that the compounds described herein can be administered to mammals other than humans for immunosuppression of mammalian autoimmune diseases.

The immunosuppressant compounds can be administered in combination with other drugs to boost the immunosuppressive effect. Compounds that can be coadministered include steroids (e.g. methyl prednisolone acetate), NSAIDS and other known immunosuppressants such as azathioprine, 15-deoxyspergualin, cyclosporin, mizoribine, mycophenolate mofetil, brequinar sodium, leflunomide, FK-506, rapamcyin and related molecules. Dosages of these drugs will also vary depending upon the condition and individual to be treated.

The assay used to measure T cell growth inhibition was a human peripheral blood lymphocyte (PBL) proliferation assay using standard procedures known in the art. PBL's were chosen due to their known ability to proliferate in the presence of antigens derived from herpes simplex virus (HSV), Rubella or tetanus toxoid (TT). PBL growth inhibition was measured in terms of the compound's ability to interfere with antigen induced lymphocyte proliferation.

The compounds of this invention can be used to produce antibodies (e.g., polyclonal and monoclonal) against the complexes. Methods for making antibodies are well known. The antibodies can be used as a diagnostic tool for monitoring the amount of the immunosuppressant compound in patient blood levels. The ability to closely monitor the amount of immunosuppressant provides a suitable means for controlling

been described for example in the following references: Inorg. Synth. 20:470 (1981); Inorg. Chem. 27:2848 (1988); Inorg. Chem. 28:3001 (1989); Inorg. Chem. 27:4587 (1988); Inorg. Chem. 24:269 (1986); Inorg. Chem. 28:1405 (1989); Inorg. Chem. 26:2667 (1987); Inorg. Chem. 27:1086 (1988); Polyhedron 5:1347 (1987); Inorg. Chem. 23:1851 (1984); Inorg. Chem. Acta 210:151 (1993); Inorg. Chem. 30:2514 (1991); Inorg. Chem. 9:1197 (1990); Inorg. Synth. 24:263 (1986); Inorg. Synth. 6:138 (1960); and Z. Anorg. Allg. Chem. 490:205 (1982).

It has now been discovered that compounds of this invention possess immunosuppressive activity as confirmed through a drug screen. Specific T cell proliferation was measured in response to antigen exposure in the presence or absence of the compounds. It was found that the compounds inhibited T cell proliferation by 50% (IC_{50}) at a concentration of about 1 to 50 μg/mL.

Compounds of this invention can be administered orally, parenterally (e.g. intramuscularly, intravenously, subcutaneously), topically, nasally or via slow releasing microcarriers in dosage formulations (e.g., therapeutically effective amount) containing a physiologically acceptable vehicle and optional adjuvants and preservatives. Suitable physiologically acceptable vehicles include saline, sterile water, creams, ointments, solutions, gels, pastes, emulsions, lotions, oils, solid carriers and aerosols.

The immunosuppressant compounds can be applied topically as a cream or ointment to locally deliver immunosuppressive concentrations of the drug without significant systemic exposure. Topical application may be the ideal way to deliver the compound for psoriasis and perhaps other inflammatory skin diseases such as contact dermatitis and pemphigus vulgaris.

carbonyl, phenyl, carboxyl, benzyl, thio and combinations of these. A preferred ligand is methylimidazole.

In another embodiment, compounds having polydentate ligands, in combination with other polydentate ligands and/or monodentate ligands, can be used in the invention. Suitable bidentate ligands (B) will include, but are not limited to, aliphatic amines (e.g., ethylenediamine (en), propylenediamine, 1,2-cyclohexanediamine and the corresponding alkylated amines thereof); heterocyclic aromatic amines (e.g., 2,2'-bipyridine (bipy), 1,10-phenanthroline); pyridine based ligands (e.g., 2-aminopicoline); pyrazole based ligands (e.g., potassium-bis-pyrazolyl borate, bis-pyrazolyl methane); carboxylates; and bis-phosphines (e.g., 1,2-bis(dimethylphosphino)methane).

Examples of tridentate ligands (T) include but are not limited to aliphatic amines (diethylenetriamine, dipropylenetriamine, 1,4,7-triazacyclononane, 1,4,7-triazacyclodecane), aromatic amines [2,2',6",2"-terpyridine, bis-(2-pyridylmethyl) amine, bis-(2-imidazolylmethyl)amine, potassium tris-pyrazolyl borate, tris-pyrazolyl methane], oxygen based ligands (iminodiacetic acid, nitrilotriacetic acid, triethanol amine, bis-(3-methylphenoxy)amine), and sulfur based ligands (1,4,7-trithiacyclononane, 1,4,7-trithiacyclodecane, 2-(arylazophenyl)thioether).

Examples of polydentate ligands (P) include but are not limited to nitrogen containing ligands [e.g., 1,4,7,10-tetrazacyclododecane; 1,4,8,11-tetraazacyclotetradecane; tris-(2-pyridylmethyl)amine; tris-(2-imidazolylmethyl) amine]; sulfur containing ligands (1,4,8,11-tetrathiacyclotetradecane, 1,4,7,10-tetrathiacyclotridecane); and phosphorus containing ligands [α,α'-bis-(bis-(2-diphenylphosphinoethyl)amino)ethane and α,α'-bis-(bis-(2-diphenylphosphino)-m-xylene].

General procedures for making compounds which are useful in this invention are well known in the art and have

the overall charge of the complex neutral and optionally to nhance solubility of the complex in a physiologically environment. The number of counterions (e.g., 1 to 5 counterions that are the sam or different from each other) will be that which is required to neutralize the overall charge of the complex. Counterions which result in physiologically acceptable salts of the complexes, including protonated salts thereof, are within the scope of this invention and include but are not limited to salts derived from inorganic cations such as sodium (Na^+), potassium (K^+), lithium (Li^+), and the like; organic bases such as mono-, di- and trialkyl amines of 1-8 carbon atoms, per alkyl group and mono-, di- and trihydroxyalkyl amines of 1-8 carbon atoms peralkyl group, and the like; and organic and inorganic acids such as acetate, lactate, citrate, tartarate, succinate, maleate, malonate, gluconate, hydrochloride, hydrobromide, phosphorate, nitrate, sulfate, trifluoromethansulfonate, methanesulfonate, and similarly known acceptable acids. Some specific examples are listed above under the definition of Z.

Examples of suitable monodentate ligands (L) comprising aliphatic and aromatic amines include but are not limited to imidazole (Im), pyridine (py), ammonia, triazole, pyrazole, quinoline, pyrazine, pyridazine, pyrimidine, quinoxaline, quinazoline, piperidine, phosphine, phosphite, thiolate, sulfoxide, nitrogen, water, halide, alkoxide, phenolate and carboxylic acids. Derivatives of these ligands can also be incorporated into the complex in various combinations with the non-substituted ligands. A derivative is a ligand in which one or more of the hydrogen atoms has been substituted with a moiety, such as C1-C8 alkyl, C3-C8 cyclic alkyl, C2-C6 alkenyl, aryl or substituted aryl, hydroxyl, carboxylate, C1-C8 linear, branched or cyclic amine, nitro, ester,

wherein R is a linear or branched alkyl (e.g., 1 to 8 carbon atoms) or aryl.

When the metal center has four coordinating atoms then it may contain four equivalent ligands (referred as monodentate) or a mixture of ligands. The mixture of ligands may contain different monodentate ligands, or a mixture of bidentate/ monodentate in a 1:2 ratio, or a mixture of bidentate ligands, or a mixture of tridentate/monodentate in a 1:1 ratio or a tetradentate ligand.

When the metal center contains six coordinating atoms then it may contain six equivalent ligands (referred as monodentate) or a mixture of different ligands. The mixture of ligands may contain monodentate ligands, or a mixture of bidentate/ monodentate ligand in a 1:4 or 2:2 ratio, or a mixture of tridentate/monodentate in a 1:3 ratio, or a mixture of tridentate/bidentate/monodentate in a 1:1:1 ratio, or a mixture of polydentate/monodentate in a 1:1 or 1:2 depending on the nature of the polydentate ligand, or a mixture of a polydentate/bidentate in a 1:1 ratio, or three bidentate ligands or two tridentate ligands. For the purposes of this application, the terms "monodentate", "bidentate", "tridentate" and "polydentate" will have their generally accepted meaning in the art. That is, a monodentate ligand is defined as a chemical moiety or group which has one potential coordinating atom. More than one potential coordinating atom is termed a polydentate ligand where the number of potential coordinating atoms is indicated by the terms bidentate, tridentate, etc. Ligands that are protonated are well within the scope of the invention.

Compounds of this invention can contain a metal center of different oxidation states, e.g., 2, 3 or 4. Depending upon the ligands, the complex can inherently be neutral, i.e., it will not require counterion(s) to neutralize the overall charge of the complex. Alternatively, the compounds can contain counterion(s) of appropriate charge to render

t is 0, 1 or 2;

p is 0 or 1;

wherein each L is independently a monodentate ligand selected from the group consisting of nitrogen containing ligands, phosphorus containing ligands, sulfur containing ligands, oxygen containing ligands, carbon containing ligands and halide (e.g., F, Cl, Br, I);

wherein each B is independently a bidentate ligand selected from the group consisting of nitrogen containing ligands (e.g., aliphatic amines, heterocyclic aromatic amines), sulfur containing ligands, carbon containing ligands, oxygen containing ligands and phosphorus containing ligands;

wherein each T is independently a tridentate ligand selected from the group consisting of nitrogen containing ligands, sulfur containing ligands, carbon containing ligands, oxygen containing ligands and phosphorus containing ligands;

wherein each P is independently a polydentate ligand selected from the group consisting of nitrogen containing ligands, oxygen containing ligands, carbon containing ligands, sulfur containing ligands and phosphorus containing ligands;

wherein when the complex is charged, then Z is at least one counterion of appropriate charge to render the overall charge of the complex neutral, e.g., a counterion selected from the group consisting of F^-, Cl^-, Br^-, I^-, NO_3^-, NH_4^+, NR_4^+, PF_6^-, BPh_4^-, SO_4^{-2}, S_8^{-2}, $S_2O_7^{-2}$, BF_4^-, H^+, $CF_3SO_3^-$, Na^+, K^+, Li^+, ClO_4^- and $RImH^+$, where Im is imidazole; and organic and inorganic salts such as acetate, lactate, citrate, tartarate, succinate, maleate, malonate, gluconate, hydrochloride, hydrobromide, phosphorate, nitrate, sulfate, trifluoromethansulfonate and methanesulfonate;

Detailed Description of the Invention

This invention is based upon the discovery that certain compounds can inhibit antigen specific T lymphocyte proliferation, *in vitro*. The data suggest that these compounds have potential use as immunosuppressants to reduce undesirable immune responses in humans. The compounds of this invention can be used to facilitate organ transplantation, and to treat human autoimmune disorders where the specific activation of T cells is responsible for, or contributes to the pathology and progression of the diseases, such as diabetes, rheumatoid arthritis, multiple sclerosis, lupus erythematosus and steroid resistant asthma.

This invention pertains to compounds that have immunosuppressive properties of the general formula:

$$[ML_lB_bT_tP_p]Z$$

and physiologically acceptable salts thereof;

wherein M is selected from the group consisting of iridium (Ir), palladium (Pd), chromium (Cr), iron (Fe), technitium (Tc), rhenium (Re), cobalt (Co), osmium (Os), manganese (Mn), copper (Cu), nickel (Ni), platinum (Pt) and rhodium (Rh), having an oxidation state of 2, 3 or 4 depending upon the nature of M;

wherein the number of coordinating atoms is 4 or 6 depending on the oxidation state and the nature of M;

wherein when the number of coordinating atoms is 4 then

l is 0, 1, 2, 3 or 4;

b is 0, 1 or 2;

t is 0 or 1;

p is 0 or 1;

wherein when the number of coordinating atoms is 6 then

l is 0, 1, 2, 3, 4, 5 or 6;

b is 0, 1, 2 or 3;

drugs, the most commonly used immunosuppressant is cyclosporin A. However, usage of cyclosporin has numerous side effects such as nephrotoxicity, hepatotoxicity and other central nervous system disorders. Thus, there is presently a need to investigate new immunosuppressive agents that are less toxic but equally as effective as those currently available.

Summary of the Invention

This invention relates to the use of compounds as immunosuppressive agents to prevent or significantly reduce graft rejection in organ and bone marrow transplantation. The compounds can also be used as an immunosuppressant drug for T lymphocyte mediated autoimmune diseases, such as diabetes, rheumatoid arthritis, multiple sclerosis, lupus erythematosus and steroid resistant asthma.

In another aspect, other diseases with suspected inflammatory components, such as psoriasis, contact dermatitis and hyperplasia of the epidermis, can be treated with the compounds of this invention to alleviate symptoms associated with these disease states.

It is known that compounds having T lymphocyte immunosuppressive properties may also be useful in inhibiting the proliferation of cardiac smooth muscle cells. Based upon this, it is expected that the compounds can be used for the treatment of hyperproliferative vascular disorders, such as restenosis and atherosclerosis.

The invention also provides a method for coadministration of the compounds described herein with agents designed for gene therapy. It is believed that the use of the compounds of this invention may suppress the immune system and reduce/minimize an immune response against the gene delivery vehicle, so that therapeutic levels of transgene expression can be achieved in an animal or human.

METAL-CONTAINING COMPOUNDS AND THEIR USE FOR INHIBITING THE IMMUNE RESPONSE

Related Applications

This is a Continuation-in-Part of U.S. Patent Application Serial Nos. 08/472,952 and 08/479,341, both filed June 7, 1995, the entire teachings of which are incorporated herein by reference.

Background of the Invention

Replacement of defective or severely injured tissues and organs has been a medical objective as long as medicine has been practiced. Grafts from an individual to himself almost invariably succeed, and are especially important in the treatment of burn patients. Likewise, grafts between two genetically identical individuals almost invariably succeed. However, grafts between two genetically dissimilar individuals would not succeed without immunosuppressive drug therapies. The major reason for their failure is a T cell mediated immune response to cell-surface antigens that distinguish donor from host.

Immunosuppressive agents are also indicated in the treatment of autoimmune diseases such as rheumatoid arthritis or type I diabetes mellitus. One particular condition worth mentioning here is psoriasis. This disease is characterized by erythematous patches of skin accompanied by discomfort and itching. Hyperplasia of the epidermis involving proliferation of keratinocytes is also a hallmark feature of psoriasis. An inflammatory component is suggested by: (i) the finding of lymphocytic infiltration of epidermis, and (ii) the fact that immunosuppressive agents such as cyclosporin and corticosteroids have beneficial effect on the disease.

A number of drugs are currently being used or investigated for their immunosuppressive properties. Among these